

What is Claimed is:

1. A polypeptide selected from the group consisting of

5 (a) a fusion polypeptide which comprises a first amino acid sequence including at least one stretch of amino acids constituting a T-cell epitope derived from the *M. tuberculosis* protein ESAT-6, and a second amino acid sequence including at least one stretch of amino acids constituting a T-cell epitope derived from the *M. tuberculosis* protein Ag85B, said first and second amino acid sequences optionally being fused via a linker sequence;

10

(b) a polypeptide comprising an amino acid sequence analogue having at least 70% sequence identity to the sequence in (a) and at the same time being immunogenic; and

(c) a fusion polypeptide which comprises a first amino acid sequence having at least 70% sequence identity to the first amino acid sequence in (a) and at the same time being immunogenic, and a second amino acid sequence having at least 70% sequence identity to the second amino acid sequence in (a) and at the same time being immunogenic, said first and second amino acid sequences optionally being fused via a linker sequence.

20

2. A polypeptide according to claim 1, wherein the degree of sequence identity is at least 75%.

3. A polypeptide according to claim 1, wherein the first amino acid sequence is situated C-terminally to the second amino acid sequence.

25

4. A polypeptide according to claim 1, wherein the first amino acid sequence is situated N-terminally to the second amino acid sequence.

5. A polypeptide according to claim 1, wherein no linkers are introduced between the two amino acid sequences in (a) or (c).

30

6. A polypeptide according to claim 1, which is Ag85B fused N- or C-terminally to ESAT-6.

7. A polypeptide according to claim 1, which is lipidated so as to allow a self-adjuvating effect of the polypeptide.

8. A polypeptide according to claim 1 for use as a vaccine or as a pharmaceutical.

9. Use of a polypeptide according to claim 1 for the preparation of a pharmaceutical

5 composition, e.g. for the vaccination against infections caused by virulent mycobacteria, e.g. by *Mycobacterium tuberculosis*, *Mycobacterium africanum* or *Mycobacterium bovis*.

10. An immunogenic composition comprising a polypeptide according to claim 1.

10 11. An immunogenic composition according to claim 10, which is in the form of a vaccine.

12. A nucleic acid fragment in isolated form which comprises a nucleic acid sequence which encodes a polypeptide as defined in claim 1, or comprises a nucleic acid sequence complementary thereto.

13. A nucleic acid fragment according to claim 12, which is a DNA fragment.

14. A nucleic acid fragment according to claim 12 for use as a pharmaceutical.

15 15. A vaccine comprising a nucleic acid fragment according to claim 12 or 13, optionally inserted in a vector, the vaccine effecting *in vivo* expression of antigen by an animal, including a human being, to whom the vaccine has been administered, the amount of expressed antigen being effective to confer substantially increased resistance to tuberculosis caused by virulent mycobacteria, e.g. by *Mycobacterium tuberculosis*, *Mycobacterium africanum* or *Mycobacterium bovis*, in an animal, including a human being.

16. Use of a nucleic acid fragment according to claim 12 or 13 for the preparation of a composition for the diagnosis of tuberculosis caused by virulent mycobacteria, e. g. by *Mycobacterium tuberculosis*, *Mycobacterium africanum* or *Mycobacterium bovis*.

17. Use of a nucleic acid fragment according to claim 12 or 13 for the preparation of a pharmaceutical composition for the vaccination against tuberculosis caused by virulent mycobacteria, e.g. by *Mycobacterium tuberculosis*, *Mycobacterium africanum* or *Mycobacterium bovis*.

18. A vaccine for immunizing an animal, including a human being, against tuberculosis caused by virulent mycobacteria, e.g. by *Mycobacterium tuberculosis*, *Mycobacterium africanum* or *Mycobacterium bovis*, comprising as the effective component a non-pathogenic microorganism, wherein at least one copy of a DNA fragment comprising a DNA sequence encoding a polypeptide according to claim 1 has been incorporated into the microorganism (e.g. placed on a plasmid or in the genome) in a manner allowing the microorganism to express and optionally secrete the polypeptide.

19. A replicable expression vector which comprises a nucleic acid fragment according to claim 12.

20. A transformed cell harbouring at least one vector according to claim 19.

21. A method for producing a polypeptide according to claim 1, comprising

- (a) inserting a nucleic acid fragment according to claim 12 into a vector which is able to replicate in a host cell, introducing the resulting recombinant vector into the host cell, culturing the host cell in a culture medium under conditions sufficient to effect expression of the polypeptide, and recovering the polypeptide from the host cell or culture medium;
- (b) isolating Ag85B and ESAT-6 from a whole mycobacterium, e.g. *Mycobacterium tuberculosis*, *Mycobacterium africanum* or *Mycobacterium bovis*, from culture filtrate or from lysates or fractions thereof, and fusing the polypeptides;
- (c) synthesizing the polypeptide e.g. by solid or liquid phase peptide synthesis; or
- (d) a combination of the methods in (a), (b) and/or (c).

22. A method for immunising an animal, including a human being, against tuberculosis caused by virulent mycobacteria, e.g. by *Mycobacterium tuberculosis*, *Mycobacterium africanum* or *Mycobacterium bovis*, comprising administering to the animal the polypeptide according to claim 1, the immunogenic composition according to claim 10, or the vaccine according to claim 18.

23. A pharmaceutical composition which comprises an immunologically responsive amount of at least one member selected from the group consisting of:

- (a) a fusion polypeptide which comprises a first amino acid sequence including at least one stretch of amino acids constituting a T-cell epitope derived from the *M. tuberculosis* protein

ESAT-6, and a second amino acid sequence including at least one stretch of amino acids constituting a T-cell epitope derived from the *M. tuberculosis* protein Ag85B, said first and second amino acid sequences optionally being fused via a linker sequence;

(b) a polypeptide comprising an amino acid sequence which has a sequence identity of at

5 least 70% to any one of said polypeptides in (a) and at the same time being immunogenic;

(c) a fusion polypeptide comprising at least one polypeptide or amino acid sequence according to (a) or (b) and at least one fusion partner;

(d) a nucleic acid sequence which encodes a polypeptide according to (a), (b) or (c);

(e) a nucleic acid sequence which is complementary to a sequence according to (d);

10 (f) a nucleic acid sequence which has a length of at least 10 nucleotides and which hybridizes under stringent conditions with a nucleic acid sequence according to (d) or (e); and
(g) a non-pathogenic micro-organism which has incorporated (e.g. placed on a plasmid or in the genome) therein a nucleic acid sequence according to (d), (e) or (f) in a manner to permit expression of a polypeptide encoded thereby.

15 24. A method for stimulating an immunogenic response in an animal which comprises administering to said animal an immunologically stimulating amount of at least one member selected from the group consisting of:

(a) a fusion polypeptide fragment which comprises a first amino acid sequence including at
20 least one stretch of amino acids constituting a T-cell epitope derived from the *M. tuberculosis* protein ESAT-6, and a second amino acid sequence including at least one stretch of amino acids constituting a T-cell epitope derived from the *M. tuberculosis* protein Ag85B, said first and second amino acid sequences optionally being fused via a linker sequence;

(b) a polypeptide comprising an amino acid sequence which has a sequence identity of at
25 least 70% to any one of said polypeptides in (a) and is immunogenic;

(c) a fusion polypeptide comprising at least one polypeptide or amino acid sequence according to (a) or (b) and at least one fusion partner;

(d) a nucleic acid sequence which encodes a polypeptide or amino acid sequence according to (a), (b) or (c);

30 (e) a nucleic acid sequence which is complementary to a sequence according to (d);

(f) a nucleic acid sequence which has a length of at least 10 nucleotides and which hybridizes under stringent conditions with a nucleic acid sequence according to (d) or (e); and

(g) a non-pathogenic micro-organism which has incorporated therein (e.g. placed on a plasmid or in the genome) a nucleic acid sequence according to (d), (e) or (f) in a manner to permit expression of a polypeptide encoded thereby.

- 5 25. Vaccine according to claim 15 or 18, immunogenic composition according to claim 10 or pharmaceutical composition according to claim 23, characterized in that said vaccine/immunogenic composition/pharmaceutical composition can be used prophylactically in a subject not infected with a virulent mycobacterium; or therapeutically in a subject already infected with a virulent mycobacterium.

10

10